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(54) Title: PHARMACEUTICAL COMPOSITION, CONTAINING FRAGMENTS OF AN ANTIGENIC PROTEIN ENCODING DNA ENDOWED WITH ANTI-TUMOR EFFECT

(57) Abstract

Provided herein is a pharmaceutical composition containing one or more DNA molecules encoding fragments of a protein overexpressed in tumor cells, in order to induce an anti-tumor Ag-specific immune response, in association with suitable excipients and adjuvants.

PHARMACEUTICAL COMPOSITION, CONTAINING FRAGMENTS OF AN ANTIGENIC
PROTEIN ENCODING DNA ENDOWED WITH ANTI-TUMOR EFFECT.

Field of the invention

5   The invention relates to a pool of DNA plasmid constructs
containing the sequences of human MUC-1 encoding fragments and
to a pool of DNA plasmids in which the fragments themselves are
preceded by the sequence encoding a protein consisting of human
ubiquitin fused to a bacterial LacI fragment. The invention
10   further   relates   to   their   use   in   the   preparation   of
pharmaceutical compositions for use as DNA anti-tumor vaccines.

Background art

The invention provides an anti-tumor therapy based on the
induction or activation of the immune response able to bring
15   about   tumor   rejection. The   validity   of   such   an   idea   is
demonstrated from the first clinical results; for example,
patients   treated   with   a   viral   vaccine   containing   the
Carcinoembryonic Antigen (CEA) encoding sequences demonstrated
immune system activation against this antigen (Tsang KY et al.
20   J. Natl. Cancer. Inst. 87: 982, 1995).

The   activation   of   an   immune   anti-tumor   response   is
achievable through four different approaches:

a) *Ex vivo* engineering of patient tumor cells in order to
make them more immunogenic and suitable as a vaccine;

25   b) *Ex vivo* engineering of patient immune cells in order to
pre-activate an *in vitro* immune response.

c) Inoculation of naked or liposome capsulated or viral
particle   integrated   (retrovirus,   vaccinia   virus,   adenovirus,
etc.) DNA encoding tumor associated antigens;

30   d) Treatment with recombinant or synthetic soluble tumor
antigens conjugated or mixed with adjuvants.

The first two approaches consist of the engineering of
every single patient cell and are limited in that they are
necessarily patient-specific, while the latter two are aimed to

obtain products comparable to a traditional drug.

The new vaccination methods reflect the development of new technologies. The recent indications coming from the experimentation on DNA naked vaccines that induce either a persistent antibody or a cell immune response, make the traditional protein subunit vaccines constituted of certain specific peptides, inducing a lymphocyte population, obsolete. Intramuscularly or intradermically injected proteins, encoded by naked DNA, induce a cytotoxic-specific response as well as a helper response. This powerful combination is extremely effective but the underling mechanism is not completely clarified yet. Muscle cells express class I MHC antigens at low levels only, and do not apparently express class II antigens or co-stimulatory molecules. Consequently, transfected muscle cells are unlikely to play an important role in the onset of the immune response per se. Recent data show that Antigen Presenting Cells (APC), such as macrophages or dendritic cells, play a fundamental role in capturing the myocyte released antigen and in the subsequent processing and presenting of the respective peptides in the context of the class I and II molecules, thus inducing a CD8+ cell activation with cytotoxic activity as well as activation of the CD4+ cells co-operating with B lymphocytes in eliciting the antibody response (*Corr M et al J. Exp. Med. 184:1555, 1996*) (*Tighe, H. et al. Immunology Today 19:89, 1998*).

Furthermore, the use of cytokines is known to improve the therapeutic effect deriving from immunization with DNA. Cytokines can be administered in the form of exogenous proteins as reported in *Irvine et al., J. Immunol. 156: 238, 1996*. An alternative approach is represented by the contemporaneous inoculation of both the tumor antigen or the desired cytokine encoding plasmids, thus allowing the cytokine to be produced *in situ* (*Kim JJ et al. Immunol 158: 816, 1997*).

The active immunization approach of the present invention is based on the use of DNA vectors as vaccines against the MUC-1

human antigen or Polymorphic Epithelial Mucin (PEM), overexpressed in tumor cells. MUC-1 is an epithelial luminal surface glycoprotein (*Patton S. et al. BBA 1241:407, 1995*). In the cell transformation process this glycoprotein loses the
5   apical localization and its expression level rises dramatically. The protein function consists of protecting the luminal surfaces, for example in the mammal gland, ovary, endometrium, colon, stomach, pancreas, bladder, kidney, etc. A glycosylation defect is reported that makes tumor cell associated MUC-1
10   antigenically different from normal cell associated MUC-1. This phenomenon causes tumor MUC-1 to expose the antigen epitopes that are normally masked by the sugar moieties in the normal cell expressed MUC-1. This characteristic makes tumor MUC-1 particularly interesting in an induction of a tumor specific
15   antibody response (*Apostolopoulos V. et al. Crit. Rev. Immunol. 14:293, 1994*).

        As an objective, the vaccination is aimed at inducing immune responses against tumor cells expressing MUC1 at high levels, preserving at the same time the low expressing normal
20   epithelia. The DNA vaccination relies upon the entrance of a gene or portions thereof inside the body cells followed by transcription and translation of the inserted sequence and thus the intracellular synthesis of the corresponding polypeptide. An important advantage of this system is that the neo-synthesized
25   protein is naturally processed inside the cell and the produced peptides are associated with the Major Histocompatibility Complex class I molecules (MHC-I). The MHC/peptide complexes are therefore naturally exported to the cell surface where they can be recognized by the immune system CD8+ cytotoxic cells. Only
30   the polypeptides synthesized inside the cell are then processed and presented in association with the MHC class I molecules, thus making it the only mechanism to stimulate, a specific cytotoxic response. Vaccination systems based on protein or peptide administration are usually more effective in stimulating

the antibody immune response which, to date, has been shown to be ineffective in rejecting tumor cells. Current gene therapy techniques rely upon DNA packaging in recombinant viral vectors (retrovirus and adenovirus). The naked DNA administration is much more advantageous in terms of effectiveness and safety compared to viral vector therapies (*Kumar V and Sercarz E. Nature Med. 2: 857, 1996; McDonnel WM et al., New England J. of Med. 334: 42, 1996*). In fact naked DNA is unable either to duplicate or integrate in the host tissue DNA and does not induce the immune response to viral proteins.

The use of the ubiquitin to enhance the neo-synthesized protein processing and thus cytotoxic lymphocyte induction was recently reported (*Rodriguez F. et al., J. Virology 71: 8497, 1997*). The use of ubiquitin in order to generate proteins with an N-terminal amino acid, making them unstable and thus prone to enhanced degradation, had been previously reported (*Bechmair A. et al., SCIENCE 234: 179, 1986*). The higher instability of these proteins was subsequently related to enhanced intracellular processing and presentation of model proteins by MHC-1 (*Grant E P et al., J. Immunol. 155: 3750, 1995*) (*Wu Y and Kipps T.J., J. Immunol. 159: 6037, 1997*).

The use of single constructs containing partial antigen encoding DNA fragments (influenza virus nucleoprotein), having a higher antigenic presentation efficiency compared to the analogues with the whole antigenic sequence, in DNA vaccination was reported (*Anton L. C. et al., J. Immunol. 158: 2535, 1997*). Furthermore the processing of intracellular proteins and presentation of the respective peptides by MHC class I proteins in physiologic conditions, underlie the mechanism of immunological surveillance. For a given protein and a specific MHC context, there are peptide fragments termed dominants (i. e. prevailing on subdominants or cryptics), which are unable to generate any immune response because they are recognized as "self". It has now been outlined, according to an aspect of the

present invention, that an approach aimed at supporting the non-dominant epitope presentation by the administration of a mix of antigen protein fragments is able to elicit a surprising cytotoxic immune response.

5   **Description of the invention**

It has now been found that DNA molecules, encoding fragments of a protein overexpressed in tumor cells, can be conveniently used to induce an antigen-specific anti-tumor immune response.

10   The invention relates particularly to a pharmaceutical composition containing one or more DNA encoding Mucin (MUC-1) protein fragments.

The DNA used in the present invention can be plasmid or viral DNA, preferably plasmid DNA obtained employing the pMRS30

15   expression vector described in fig. 13.

The compositions according to the invention contain preferably at least two DNA fragments of the Mucin (MUC-1) or of another protein overexpressed in tumor cells.

The compositions according to the invention contain

20   preferably at least four fragments, each ranging from 200 to about 700 nucleotides, each sequence being juxtaposed and possibly partially overlapping, from about 50 to about 150 nucleotides, at the 3' and/or 5' end of the adjacent one.

The DNA fragments according to the invention can be

25   possibly preceded at the 5' end by a ubiquitin encoding DNA sequence and possibly also by a LacI portion of Escherichia coli.

The invention relates also to new DNA fragments and to the use of Mucin-1 fragments defined above in the medicine and anti-

30   tumor vaccine preparation.

Description of the figures

Fig. 1

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS166 expression

vector. This DNA includes the sequence corresponding to nucleotides 136-339 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by the two translation stop codons, TGA and TAA. The encoded polypeptide thus includes a Metionin followed by the amino acids encoded by the 136-339 fragment of the EMBL sequence J05581.

Fig. 2

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS169 expression vector. This DNA includes the sequence corresponding to nucleotides 205-720 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes a Metionin followed by the amino acids encoded by the 205-720 fragment of the EMBL sequence J05581.

Fig. 3

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS168 expression vector. This DNA includes the sequence corresponding to nucleotides 631-1275 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes a Metionin followed by the amino acids encoded by the 631-1275 fragment of the EMBL sequence J05581.

Fig. 4

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS167 expression vector. This DNA includes the sequence corresponding to nucleotides 1222-1497 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes a Metionin followed by the

amino acids encoded by the 1222-1497 fragment of the EMBL sequence J05581.

Fig. 5

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS175 expression vector. This DNA includes the sequence corresponding to nucleotides 136-1497 of the EMBL sequence J05581, preceded by the translation start codon, ATG and followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes a Metionin followed by the amino acids encoded by the 136-1497 fragment of the EMBL sequence J05581.

Fig. 6

Nucleotide DNA sequence (with the respective amino acid sequence) termed UBILacI. The encoded polypeptide includes the Ubiquitin sequence fused to a partial sequence of the bacterial protein beta-galactosidase, as described in *Chau V. et al. Science 243: 1576, 1989.*

Fig. 7

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the expression vector pMRS30 to give the pMRS171 expression vector. This DNA includes the sequence termed UBILacI (see fig. 6) fused to the sequence corresponding to nucleotides 136-339 of the EMBL sequence J05581 followed by two translation stop codons, TGA and TAA. The coded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 136-339 of the EMBL sequence J05581.

Fig. 8

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS174 expression vector. This DNA includes the sequence termed UBILacI (see fig. 6) fused to the sequence partially corresponding to nucleotides 205-720 of the EMBL

sequence J05581 followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 205-720 of the EMBL sequence J05581.

Fig. 9

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS173 expression vector. This DNA includes the sequence termed UBILacI (see fig. 6) fused to the sequence partially corresponding to nucleotides 631-1275 of the EMBL sequence J05581 followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 631-1275 of the EMBL sequence J05581.

Fig. 10

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS172 expression vector. This DNA includes the sequence termed UBILacI (see fig. 6) fused to the sequence partially corresponding to nucleotides 1222-1497 of the EMBL sequence J05581 followed by two translation stop codons, TGA and TAA. The encoded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 1222-1497 of the EMBL sequence J05581.

Fig. 11

Nucleotide DNA sequence (with the respective amino acid sequence) inserted at the XbaI site of the pMRS30 expression vector to give the pMRS176 expression vector. This DNA includes the sequence named UBILacI (see fig. 6) fused to the sequence partially corresponding to nucleotides 136-1497 of the EMBL sequence J05581 followed by two translation stop codons, TGA and

TAA. The encoded polypeptide thus includes the amino acid sequence reported in Fig. 6, fused to the sequence including the amino acids encoded by the fragment 136-1497 of the EMBL sequence J05581.

Fig. 12

Electrophoretic analysis on 1% agarose gel in 1X TBE. mRNA extracted from CHO, CD34+ dendritic cells and dendritic cells from PBMC, respectively, transfected with pMRS169, and subjected to RT-PCR reaction either with (lanes 4, 8, 12) or without (lanes 5, 9, 13) Reverse Transcriptase. Molecular weight DNA marker (lane 1); internal negative controls (lanes 2, 6); internal positive controls (lanes 3, 7, 10, 11); positive control from Promega kit (lane 14).

Fig. 13

Nucleotide sequence of the pMRS30 expression vector. The 1-2862 region corresponds to the AccI (location 504) - BamHI (location 3369) region of the pSV2CAT vector (EMBL M77788); the 2863-3721 region includes the human cytomegalovirus promoter (human cytomegalovirus major immediate-early gene enhancer); the 3722-4905 region includes several cloning sites, including XbaI (location 3727), and the processing signal of the rabbit beta-globin gene.

<u>Detailed description of the invention</u>

A DNA plasmid pool encoding, in eukaryotic cells, fragments of the MUC-1 human protein antigen was prepared. Constructs are based on the mammalian expression vector termed pMRS30, described in figure 13 and previously claimed in the Patent Application WO95/11982, and contain partial sequences of the MUC-1 cDNAs reported in the EMBL database with accession number J05581. MUC-1 encoding DNA was fragmented so that each fragment represents a discrete portion, partially overlapping to the adjacent ones. Administration of a mix of such plasmids can cause different plasmids to transfect different APC cells at the administration site. Therefore such cells produce and process

discrete portions of the MUC-1 protein giving the related peptides. In those conditions, the occurring subdominant and cryptic peptides can also be presented in association with class I MHC molecules thus generating a cytotoxic immune response.

The present invention thus relates to the use of a group of four constructs (Figures 1 to 4) containing MUC-1 cDNA partial fragments in admixture containing at least two of them and a group of four constructs (Figures 7 to 10) containing MUC-1 cDNA partial fragment preceded by the DNA encoding a protein sequence containing Ubiquitin and an Escherichia coli Lac I portion (Figure 6) used separately or in admixture containing at least two of them.

The present invention relates also to the use of the construct (Figure 5) containing the almost complete sequence of the MUC-1 cDNA and the construct (Figure 11) containing the almost complete sequence of the MUC-1 cDNA preceded by the DNA encoding a protein sequence containing Ubiquitin and an Escherichia coli Lac I portion.

The mixture of the four constructs containing the partial fragments of the MUC-1 cDNA and the mixture of the four constructs containing the partial fragments of the MUC-1 cDNA preceded by the DNA encoding a protein sequence, containing Ubiquitin and an Escherichia coli Lac I portion, represents a preferred embodiment of the present invention.

Constructs according to the present invention can be used in the anti-tumor therapy of patient affected with tumors characterized by high MUC-1 expression.

Constructs described in the present invention were obtained as follows.

In the case of the first series of constructs, the fragments of the MUC-1 DNA were obtained by RT-PCR from BT20 cell line or by DNA partial chemical synthesis. Such fragments were then cloned into the pMRS30 expression vector and verified by sequencing.

In the case of the second series of constructs, the fragments were obtained from the first series of constructs by a PCR re-amplification. These fragments were then fused to the DNA encoding the Ubiquitin (obtained by RT-PCR from MCF7 cell line mRNA) and a partial lacI sequence (obtained by PCR from the commercial vector pGEX). DNA sequences thus obtained were then cloned in the pMRS30 expression vector and verified by sequencing. For the intended therapeutic or prophylactic uses, fragments or constructs according to the invention are suitably formulated, using carriers and methods previously employed in naked DNA vaccines, as described for example in The Immunologist, 1994, 2:1; WO 90/11092, Proc. Natl. Acad. Sci. U.S.A., 1986, 83, 9551; US 5580859; Immunology today 19 (1998), 89-97); Proc. Natl. Acad. Sci. U.S.A. 90 (1993), 11478-11482; Nat. Med. 3 (1997), 526-532; Vaccine 12 (1994), 1495-1498; DNA Cell. Biol. 12 (1993), 777-783. The dosages will be determined on the basis of clinical and pharmacological-toxicological trials. Generally speaking, they will be comprised between 0.005 µg/kg and 5 µg/kg of the fragment mix. The composition of the invention can also contain a cytokine or a cytokine encoding plasmid.

The invention will be further illustrated by means of the following examples.

Example 1. Plasmid pMRS166 construction.

BT20 tumor cells (ATCC HTB-19) were cultured in Eagles MEM supplemented with 10% fetal calf serum. Ten million cells were trypsinized, washed with PBS, and mRNA extracted.

An aliquot of this RNA was subjected to RT-PCR (reverse transcriptase-polymerase chain reaction) reaction in the presence of the following synthetic oligonucleotides:

V11 (5 GATCTCTAGAATGACAGGTTCTGGTCATGCAAGC 3)

V4 (5 GATCTCTAGAAAGCTTATCAACCTGAAGCTGGTTCCGTGGC 3)

The produced DNA fragment, purified and digested with the restriction enzyme XbaI, was cloned into the pMRS30 expression

vector, containing the human cytomegalovirus promoter and the beta-globin polyadenylation signal as claimed in the Patent WO9511982. The resulting pMRS166 vector contains a DNA fragment including the ATG codon, the sequence corresponding to the nucleotides 136-339 of the EMBL sequence J05581, and two stop codons, TGA and TAA.

This fragment is reported in fig. 1.

Example 2. Plasmid pMRS169 construction.

An aliquot of the RNA obtained as reported in example 1 was amplified by RT-PCR in the presence of the following synthetic oligonuclotides:

V12 (5 GATCTCTAGAATGGTGCCCAGCTCTACTGAGAAGAATGC 3)

V15 (5 GGCGGTGGAGCCCGGGGCTGGCTTGT 3)

The produced DNA fragment, purified and digested with the restriction enzymes SmaI and XbaI, was fused, by the SmaI restriction site, to a DNA fragment entirely synthetically constructed, and including a sequence partially corresponding to the nucleotides 457-720 of the EMBL sequence J05581 and two stop codons, TGA and TAA. The whole fragment was thus cloned in the XbaI site of the pMRS30 expression vector. The resulting pMRS169 vector contains a DNA fragment including the ATG codon, the sequence partially corresponding to the nucleotides 205-720 of the EMBL sequence J05581, and two stop codons, TGA and TAA.

This fragment is reported in fig. 2.

Example 3. Plasmid pMRS168 construction.

An aliquot of the RNA obtained as reported in example 1 was amplified by RT-PCR in the presence of the following synthetic oligonuclotides:

V13 (5 GATCTCTAGAATGGGCTCAGCTTCTACTCTGGTGCACAACGGC 3)

V8   (5 GATCTCTAGAAAGCTTATCACAAGGCAATGAGATAGACAATGGCC 3)

The produced DNA fragment, purified and digested with the restriction enzyme XbaI was cloned in the pMRS30 expression vector. The resulting pMRS168 vector contains a DNA fragment including the ATG codon, the sequence corresponding to the

nucleotides 631-1275 of the EMBL sequence J05581, and two stop codons, TGA and TAA.

This fragment is reported in fig. 3.

Example 4. Plasmid pMRS167 construction.

An aliquot of the RNA obtained as reported in example 1 was subjected to RT-PCR reaction in the presence of the following synthetic oligonucleotides:

V14 (5 GATCTCTAGAATGCTGGTGCTGGTCTGTGTTCTGGTTGCGC 3)

V10 (5 GATCTCTAGAAAGCTTATCACAAGTTGGCAGAAGTGGCTGC 3)

The produced DNA fragment, purified and digested with the restriction enzyme XbaI was cloned in the pMRS30 expression vector. The resulting pMRS167 vector contains a DNA fragment including the ATG codon, the sequence corresponding to the nucleotides 1222-1497 of the EMBL sequence J05581, and two stop codons, TGA and TAA.

This fragment is reported in fig. 4.

Example 5. Plasmid pMRS175 construction.

pMRS166, 169, 168, 167 plasmids were subjected to PCR reaction in the presence of the following nucleotide pairs:

V11 (see example 1)

V18 (5 AACCTGAAGCTGGTTCCGTGGC 3) for pMRS166

V19 (5 GTGCCCAGCTCTACTGAGAAGAATGC 3)

V20 (5 GCTGGGAATTGAGAATGGAGTGCTCTTGC 3) for pMRS169

V21 (5 GGCTCAGCTTCTACTCTGGTGCACAACGGC 3)

V22 (5 CAAGGCAATGAGATAGACAATGGCC 3) for pMRS168

V23 (5 CTGGTGCTGGTCTGTGTTCTGGTTGCG 3)

V10 (see example 4) for pMRS167

The four DNA fragments obtained in the respective PCR reactions were mixed in equimolar amounts and PCR reacted in the presence of the V11 and V10 oligonuclotides.

The produced DNA fragment, purified and digested with the XbaI restriction enzyme, was cloned in the pMRS30 expression vector. The resulting pMRS175 vector contains a DNA fragment including the ATG codon, the sequence partially corresponding to

the nucleotides 136-1497 of the EMBL sequence J05581 and two stop codons TGA and TAA.

This fragment is reported in fig. 5.

Example 6. Plasmid pMRS171 construction.

MCF7 tumor cells (ATCC HTB-22) were cultured in Eagles MEM supplemented with 10% fetal calf serum. Ten million cells were trypsinized, washed with PBS, and mRNA extracted.

An aliquot of this RNA was subjected to RT-PCR in the presence of the following synthetic oligonucleotides:

UBIup (5GATCTCTAGAATGCAGATCTTCGTGAAGACCCTGACTGGT 3)

UBIdown

(5TCACCAGCGAGACGGGCAACAGCCATGCACCACTACCGTGCCTCCCACCTCTGAGACGGAGC ACCAGG 3)

The reaction produces a DNA fragment termed fragment 1.

DNA from pGEX11T (Pharmacia) was subjected to PCR reaction in the presence of the following synthetic oligonucleotides:

LacIup (5CCTCCGTCTCAGAGGTGGGAGGCACGGTAGTGGTGCATGGCTGTTGCCC GTCTCGCTGGTGAAAAG 3)

LacIdown (5GATCGGATCCTCGGGAAACCTGTCGTGCCAGCTGC 3)

This reaction gives a DNA fragment termed fragment 2.

The 1 and 2 DNA fragments, obtained in the respective PCR reactions, were mixed in equimolar amounts and subjected to PCR reaction in presence of the UBIup and LacIdown oligonucleotides.

The produced DNA fragment, purified and digested with the restriction enzymes XbaI and BamHI, was cloned into the pUC18 commercial plasmid. The resulting pMRS156 vector contains a DNA fragment including the sequence encoding the ubiquitin fused to the sequence encoding a bacterial beta-galactosidase portion. This fragment, termed UBILacI, is reported in fig. 6.

Plasmid pMRS166 DNA was subjected to a PCR reaction in presence of the following synthetic oligonucleotides:

V3 (5GATCGGATCCACAGGTTCTGGTCATGCAAGC 3)

V4 (see Example 1)

The produced DNA fragment, purified and digested with the

restriction enzymes XbaI and BamHI, was fused, by ligation into
the two BamHI sites, to the UBILacI fragment deriving from the
pMRS156 plasmid. The resulting fragment was cloned into the
pMRS30 expression vector. The resulting pMRS171 vector contains
a DNA fragment including the UBILacI sequence, the sequence
corresponding to the 136-339 nucleotides of the EMBL sequence
J05581 and two stop codons, TGA and TAA. This fragment is
reported in fig. 7.

Example 7. Plasmid pMRS174 construction.

Plasmid pMRS169 DNA was subjected to PCR reaction in the
presence of the following synthetic oligonucleotides:

V5 (5GATCGGATCCGTGCCCAGCTCTACTGAGAAGAATGC 3)

V6 (5GATCTCTAGAAAGCTTATCAGCTGGGAATTGAGAATGGAGTGCTCTTGC 3)

The produced DNA fragment, purified and digested with the
restriction enzymes XbaI and BamHI, was fused, by ligation into
the two BamHI sites, to the UBILacI fragment deriving from the
pMRS156 plasmid. The resulting fragment was cloned into the
pMRS30 expression vector. The resulting pMRS174 vector contains
a DNA fragment including the UBILacI sequence, the sequence
corresponding to the 205-720 nucleotides of the EMBL sequence
J05581, and two stop codons, TGA and TAA. This fragment is
reported in fig. 8.

Example 8. Plasmid pMRS173 construction.

Plasmid pMRS168 DNA was subjected to PCR reaction in the
presence of the following synthetic oligonucleotides:

V7 (5GATCGGATCCGGCTCAGCTTCTACTCTGGTGCACAACGGC 3)

V8 (see example 3)

The produced DNA fragment, purified and digested with the
restriction enzymes XbaI and BamHI, was fused, by ligation into
the two BamHI sites, to the UBILacI fragment deriving from the
pMRS156 plasmid. The resulting fragment was cloned into the
pMRS30 expression vector. The resulting pMRS173 vector contains
a DNA fragment including the UBILacI sequence, the sequence
corresponding to the 631-1275 nucleotides of the EMBL sequence

J05581, and two stop codons, TGA and TAA. This fragment is reported in fig. 9.

Example 9. Plasmid pMRS172 construction.

Plasmid pMRS167 DNA was subjected to PCR reaction in the presence of the following synthetic oligonucleotides:

V9 (5 GATCGGATCCCTGGTGCTGGTCTGTGTTCTGGTTGCGC 3)

V10 (see example 4)

The produced DNA fragment, purified and digested with the restriction enzymes XbaI and BamHI, was fused, by ligation into the two BamHI sites, to the UBILacI fragment deriving from pMRS156 plasmid. The resulting fragment was cloned into the pMRS30 expression vector. The resulting pMRS172 vector contains a DNA fragment including the UBILacI sequence, the sequence corresponding to the 1222-1497 nucleotides of the EMBL sequence J05581, and two stop codons, TGA and TAA. This fragment is reported in fig. 10.

Example 10. Plasmid pMRS176 construction.

Plasmid pMRS167 DNA was subjected PCR reaction in the presence of the following synthetic oligonucleotides:

V3 (see example 6)

V10 (see example 4)

The produced DNA fragment, purified and digested with the restriction enzymes XbaI and BamHI, was fused, by ligation into the two BamHI sites, to the UBILacI fragment deriving from pMRS156 plasmid. The resulting fragment was cloned into the pMRS30 expression vector. The resulting pMRS176 vector contains a DNA fragment including the UBILacI sequence, the sequence corresponding to the 136-1497 nucleotides of the EMBL sequence J05581, and two stop codons, TGA and TAA. This fragment is reported in fig. 11.

Example 11. Eukaryotic cell transfection and testing for transcription.

CHO (Chinese Hamster Ovary) cells were cultured in alpha MEM supplemented with ribonucleotides and deoxyribonucleotides

at transfection time.

Dendritic cells were obtained from CD34+ hemopoietic precursors cultured in IMDM without serum, supplemented with GM-CSF, IL4, SCF, Flt3 and TNFalpha. After 7 days the obtained cell population was transfected.

Dendritic cells were obtained from monocytes isolated from PBMC (peripheral blood mononuclear cells), cultured in RPMI supplemented with FCS, GM-CSF, and IL-4. After 7 days the obtained cell population was transfected.

In each case, about one million cells were transfected with one of the plasmids reported in examples 1 to 10. Transfection was carried out using 3 µg of plasmid DNA and 4 µl of DMRIE (Gibco) by lipofection.

After 24 hours cells were harvested, washed with PBS and lysed in order to extract the mRNA.

A mRNA aliquot was subjected to RT-PCR reaction in the presence of the oligonucleotide pair specific for the transfected DNA plasmid.

This experiment was carried out for each plasmid reported in the examples 1 to 10, using the following oligonucleotide pairs: V11/V4 for pMRS166, V12/V6 for pMRS169, V13/V8 for pMRS168, V4/V10 for pMRS167, V4/V10 for pMRS175, UBIup/V4 for pMRS171, UBIup/V6 for pMRS174, UBIup/V8 for pMRS173, UBIup/V10 for pMRS172, V14/V10 for pMRS176.

As a representative example, figure 12 reports the electrophoretic analysis of the DNA fragments obtained by RT-PCR from the mRNA of the three cell populations, transfected with the pMRS169 plasmid. In this case the oligonucleotide pair V12/V6 was used.

Example 12. *In vivo* study results.

In the *in vivo* studies, the mixtures of the four fragments and the pMRS30 plasmid (vector without insert and thus used as a negative control) were used. In order to test the occurred immunization, an ELISA test was used to show the human mucin

specific antigens.

The *in vivo* studies were conducted using human MUC1 transgenic C57BL mice. As a consequence in these animals the MUC1 protein represents a self-protein. The employed vaccination schedule consists of 3 intradermic (dorsal portion, 50 micrograms DNA for each side) administrations (at days 0, 14, 28) of 100 micrograms plasmid DNA. At day 14 after the last administration, the animals were sacrificed and sera were tested for anti-human mucin antibodies.

The assayed fragment mixes, object of the present invention, stimulated a good immune response in the treated animals.

On the other hand, vaccination experiments with a 60-aminoacid peptide corresponding to the 20 aminoacids reported in fig. 2, from location 86 to location 105, repeated three times (this peptide is termed 3XTR), were also carried out.

The two vaccinations differ in the type of the elicited antibody response. The antibody titer results much more higher in the vaccination with 3XTR. Furthermore the noticed IgG subtypes are in favor of an essentially humoral (antibody) response in the case of vaccination with 3XTR, and of a cellular response (cytotoxic) in the case of vaccination with DNA. For anti-tumor therapy, a principally cytotoxic immune response is preferable. Because the experiments were carried out on transgenic mice, in whom the human mucin is "self", we can foresee a similar response in humans. This response could justify the use, as DNA vaccines, of the compounds of the present invention in the treatment of MUC1 overxpressing human tumors.

<u>CLAIMS</u>

1. Pharmaceutical composition containing one or more DNA molecules, encoding fragments of a protein overexpressed in tumor cells in order to induce an antitumor Ag-specific immune response, in combination with suitable excipients and adjuvants.

2. Pharmaceutical composition according to claim 1 wherein the overexpressed protein is MUC-1.

3. Pharmaceutical composition according to claim 1 or 2 containing at least two DNA molecules each containing a cDNA sequence encoding a Mucin fragment (MUC-1).

4. Composition according to claim 3 containing at least three DNA molecules each containing a cDNA sequence encoding a Mucin fragment (MUC-1).

5. Composition according to claim 4 containing at least four DNA molecules each containing a cDNA sequence encoding a Mucin fragment (MUC-1).

6. Composition according to claims 3, 4 or 5 wherein the DNA sequences comprise about 200 to about 700 nucleotides, each sequence being contiguous and possibly partially overlapping, from about 50 to about 150 nucleotides at the 3' and/or 5' end, to the adjacent one.

7. Pharmaceutical composition according to any claim from 2 to 6 wherein the used mixture consists of, at least, two plasmid DNA molecules, each containing a DNA fragment selected from those whose sequences are described in figures 1, 2, 3, and 4.

8. Pharmaceutical composition according to claim 7 wherein the used mixture consists of the pool of plasmid DNA molecules, where each molecule contains a DNA fragment selected from those whose sequences are described in figures 1, 2, 3, and 4.

9. Pharmaceutical composition according to claim 1 or 2 wherein a plasmid DNA molecule containing the sequence described in figure 5 is used.

10. Pharmaceutical composition according to claims 7, 8, or 9

wherein the used plasmid DNA molecules derive from the fusion of the pMRS30 expression vector in Fig. 13 to each sequence described in figures 1, 2, 3, 4, 5.

11. Pharmaceutical composition according to claims 2 to 6 wherein the used sequences, corresponding to single fragments of the protein, are preceded in the 5' termini by the sequence described in Fig. 6 encoding the ubiquitin and a LacI portion from Escherichia Coli.

12. Pharmaceutical composition according to claim 11 wherein the mixture consists of one or more sequences deriving from joining the pMRS30 expression vector, described in Fig. 13, to a DNA sequence selected from those described in figures 7, 8, 9, and 10.

13. Pharmaceutical composition according to claim 11 wherein the mixture consists of the totality of the sequences deriving from joining the pMRS30 expression vector to a DNA sequence selected from those described in figures 7, 8, 9, and 10.

14. Pharmaceutical composition according to claim 11 wherein the mixture consists of a sequence deriving from joining the pMRS30 expression vector to the sequence described in figure 11.

15. Pharmaceutical composition according to any preceding claims, further containing a cytokine or a cytokine encoding plasmid.

16. A plasmid DNA molecule consisting of the pMRS30 expression vector joined to a DNA sequence, encoding a MUC-1 protein fragment and whose sequence is selected from the group of those described in figures 1, 2, 3, 4, and 5.

17. A DNA molecule encoding a protein MUC-1 fragment preceded in its 5' terminus by the sequence described in Fig. 6.

18. A DNA molecule according to claim 17 selected from those described in figures 7, 8, 9, 10, and 11.

19. A plasmid DNA molecule obtained by joining the pMRS expression vector to a DNA molecule selected from those of claim 17 or 18.

20. Use of DNA molecules of claims 16-19 in the preparation of a composition with anti-tumor effect.

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Figure 1

```
  1  ATGACAGGTTCTGGTCATGCAAGCTCTACCCCAGGTGGAGAAAAG
  1▶ Met Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly Gly Glu Lys
 46  GAGACTTCGGCTACCCAGAGAAGTTCAGTGCCCAGCTCTACTGAG
 16▶ Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser Thr Glu
 91  AAGAATGCTGTGAGTATGACCAGCAGCGTACTCTCCAGCCACAGC
 31▶ Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His Ser
136  CCCGGTTCAGGCTCCTCCACCACTCAGGGACAGGATGTCACTCTG
 46▶ Pro Gly Ser Gly Ser Ser Thr Thr Gln Gly Gln Asp Val Thr Leu
181  GCCCCGGCCACGGAACCAGCTTCAGGTTGATAA
 61▶ Ala Pro Ala Thr Glu Pro Ala Ser Gly • • • • • •
```

Figure 2

```
  1  ATGGTGCCCAGCTCTACTGAGAAGAATGCTGTGAGTATGACCAGC
  1▶ Met Val Pro Ser Ser Thr Glu Lys Asn Ala Val Ser Met Thr Ser
 46  AGCGTACTCTCCAGCCACAGCCCCGGTTCAGGCTCCTCCACCACT
 16▶ Ser Val Leu Ser Ser His Ser Pro Gly Ser Gly Ser Ser Thr Thr
 91  CAGGGACAGGATGTCACTCTGGCCCCGGCCACGGAACCAGCTTCA
 31▶ Gln Gly Gln Asp Val Thr Leu Ala Pro Ala Thr Glu Pro Ala Ser
136  GGTTCAGCTGCCACCTGGGGACAGGATGTCACCTCGGTCCCAGTC
 46▶ Gly Ser Ala Ala Thr Trp Gly Gln Asp Val Thr Ser Val Pro Val
181  ACCAGGCCAGCCCTGGGCTCCACCACCCCGCCAGCCCACGATGTC
 61▶ Thr Arg Pro Ala Leu Gly Ser Thr Thr Pro Pro Ala His Asp Val
226  ACCTCAGCCCCGGACAACAAGCCAGCCCCGGGAAGTACTGCTCCA
 76▶ Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro Gly Ser Thr Ala Pro
271  CCAGCACACGGTGTTACCTCGGCTCCGGATACCAGGCCGGCCCCA
 91▶ Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro
316  GGTAGTACCGCCCCTCCTGCCCATGGTGTCACATCTGCCCCGGAC
106▶ Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp
361  AACAGGCCTGCATTGGGTAGTACAGCACCGCCAGTACACAACGTT
121▶ Asn Arg Pro Ala Leu Gly Ser Thr Ala Pro Pro Val His Asn Val
406  ACTAGTGCCTCAGGCTCTGCTAGCGGCTCAGCTTCTACTCTGGTG
136▶ Thr Ser Ala Ser Gly Ser Ala Ser Gly Ser Ala Ser Thr Leu Val
451  CACAACGGCACCTCTGCGCGCGCGACCACAACCCCAGCGAGCAAG
151▶ His Asn Gly Thr Ser Ala Arg Ala Thr Thr Thr Pro Ala Ser Lys
496  AGCACTCCATTCTCAATTCCCAGCTGATAA
166▶ Ser Thr Pro Phe Ser Ile Pro Ser • • • • • •
```

Figure 3

```
   1  ATGGGCTCAGCTTCTACTCTGGTGCACAACGGCACCTCTGCCAGG
   1▶ MetGlySerAlaSerThrLeuValHisAsnGlyThrSerAlaArg
  46  GCTACCACAACCCCAGCCAGCAAGAGCACTCCATTCTCAATTCCC
  16▶ AlaThrThrThrProAlaSerLysSerThrProPheSerIlePro
  91  AGCCACCACTCTGATACTCCTACCACCCTTGCCAGCCATAGCACC
  31▶ SerHisHisSerAspThrProThrThrLeuAlaSerHisSerThr
 136  AAGACTGATGCCAGTAGCACTCACCATAGCACGGTACCTCCTCTC
  46▶ LysThrAspAlaSerSerThrHisHisSerThrValProProLeu
 181  ACCTCCTCCAATCACAGCACTTCTCCCCAGTTGTCTACTGGGGTC
  61▶ ThrSerSerAsnHisSerThrSerProGlnLeuSerThrGlyVal
 226  TCTTTCTTTTTCCTGTCTTTTCACATTTCAAACCTCCAGTTTAAT
  76▶ SerPhePhePheLeuSerPheHisIleSerAsnLeuGlnPheAsn
 271  TCCTCTCTGGAAGATCCCAGCACCGACTACTACCAAGAGCTGCAG
  91▶ SerSerLeuGluAspProSerThrAspTyrTyrGlnGluLeuGln
 316  AGAGACATTTCTGAAATGTTTTTGCAGATTTATAAACAAGGGGGT
 106▶ ArgAspIleSerGluMetPheLeuGlnIleTyrLysGlnGlyGly
 361  TTTCTGGGCCTCTCCAATATTAAGTTCAGGCCAGGATCTGTGGTG
 121▶ PheLeuGlyLeuSerAsnIleLysPheArgProGlySerValVal
 406  GTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCAATGTCCAC
 136▶ ValGlnLeuThrLeuAlaPheArgGluGlyThrIleAsnValHis
 451  GACGTGGAGACACAGTTCAATCAGTATAAAACGGAAGCAGCCTCT
 151▶ AspValGluThrGlnPheAsnGlnTyrLysThrGluAlaAlaSer
 496  CGATATAACCTGACGATCTCAGACGTCAGCGTGAGTGATGTGCCA
 166▶ ArgTyrAsnLeuThrIleSerAspValSerValSerAspValPro
 541  TTTCCTTTCTCTGCCCAGTCTGGGGCTGGGGTGCCAGGCTGGGGC
 181▶ PheProPheSerAlaGlnSerGlyAlaGlyValProGlyTrpGly
 586  ATCGCGCTGCTGGTGCTGGTCTGTGTTCTGGTTGCGCTGGCCATT
 196▶ IleAlaLeuLeuValLeuValCysValLeuValAlaLeuAlaIle
 631  GTCTATCTCATTGCCTTGTGATAA
 211▶ ValTyrLeuIleAlaLeu••••••
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Figure 4

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  1  ATGCTGGTGCTGGTCTGTGTTCTGGTTGCGCTGGCCATTGTCTAT
  1▶ Met Leu Val Leu Val Cys Val Leu Val Ala Leu Ala Ile Val Tyr
 46  CTCATTGCCTTGGCTGTCTGTCAGTGCCGCCGAAAGAACTACGGG
 16▶ Leu Ile Ala Leu Ala Val Cys Gln Cys Arg Arg Lys Asn Tyr Gly
 91  CAGCTGGACATCTTTCCAGCCCGGGATACCTACCATCCTATGAGC
 31▶ Gln Leu Asp Ile Phe Pro Ala Arg Asp Thr Tyr His Pro Met Ser
136  GAGTACCCCACCTACCACACCCATGGGCGCTATGTGCCCCCTAGC
 46▶ Glu Tyr Pro Thr Tyr His Thr His Gly Arg Tyr Val Pro Pro Ser
181  AGTACCGATCGTAGCCCCTATGAGAAGGTTTCTGCAGGTAATGGT
 61▶ Ser Thr Asp Arg Ser Pro Tyr Glu Lys Val Ser Ala Gly Asn Gly
226  GGCAGCAGCCTCTCTTACACAAACCCAGCAGTGGCAGCCACTTCT
 76▶ Gly Ser Ser Leu Ser Tyr Thr Asn Pro Ala Val Ala Ala Thr Ser
271  GCCAACTTGTGATAA
 91▶ Ala Asn Leu••••••
```

Figure 5

```
  1  ATGACAGGTTCTGGTCATGCAAGCTCTACCCCAGGTGGAGAAAAG
  1▶ MetThrGlySerGlyHisAlaSerSerThrProGlyGlyGluLys
 46  GAGACTTCGGCTACCCAGAGAAGTTCAGTGCCCAGCTCTACTGAG
 16▶ GluThrSerAlaThrGlnArgSerSerValProSerSerThrGlu
 91  AAGAATGCTGTGAGTATGACCAGCAGCGTACTCCAGCCACAGC
 31▶ LysAsnAlaValSerMetThrSerSerValLeuSerSerHisSer
136  CCCGGTTCAGGCTCCTCCACCACTCAGGGACAGGATGTCACTCTG
 46▶ ProGlySerGlySerSerThrThrGlnGlyGlnAspValThrLeu
181  GCCCCGGCCACGGAACCAGCTTCAGGTTCAGCTGCCACCTGGGGA
 61▶ AlaProAlaThrGluProAlaSerGlySerAlaAlaThrTrpGly
226  CAGGATGTCACCTCGGTCCCAGTCACCAGGCCAGCCCTGGGCTCC
 76▶ GlnAspValThrSerValProValThrArgProAlaLeuGlySer
271  ACCACCCCGCCAGCCCACGATGTCACCTCAGCCCCGGACAACAAG
 91▶ ThrThrProProAlaHisAspValThrSerAlaProAspAsnLys
316  CCAGCCCCGGGAAGTACCGCTCCACCAGCACACGGTGTTACCTCG
106▶ ProAlaProGlySerThrAlaProProAlaHisGlyValThrSer
361  GCTCCGGATACCAGGCCGGCCCCAGGTAGTACCGCCCCTCCTGCC
121▶ AlaProAspThrArgProAlaProGlySerThrAlaProProAla
406  CATGGTGTCACATCTGCCCCGGACAACAGGCCTGCATTGGGTAGT
136▶ HisGlyValThrSerAlaProAspAsnArgProAlaLeuGlySer
451  ACAGCACCGCCAGTACACAACGTTACTAGTGCCTCAGGCTCTGCT
151▶ ThrAlaProProValHisAsnValThrSerAlaSerGlySerAla
496  AGCGGCTCAGCTTCTACTCTGGTGCACAACGGCACCTCTGCGCGC
166▶ SerGlySerAlaSerThrLeuValHisAsnGlyThrSerAlaArg
541  GCGACCACAACCCCAGCGAGCAAGAGCACTCCATTCTCAATTCCC
181▶ AlaThrThrThrProAlaSerLysSerThrProPheSerIlePro
586  AGCCACCACTCTGATACTCCTACCACCCTTGCCAGCCATAGCACC
196▶ SerHisHisSerAspThrProThrThrLeuAlaSerHisSerThr
631  AAGACTGATGCCAGTAGCACTCACCATAGCACGGTACCTCCTCTC
211▶ LysThrAspAlaSerSerThrHisHisSerThrValProProLeu
676  ACCTCCTCCAATCACAGCACTTCTCCCCAGTTGTCTACTGGGGTC
226▶ ThrSerSerAsnHisSerThrSerProGlnLeuSerThrGlyVal
721  TCTTTCTTTTTCCTGTCTTTTCACATTTCAAACCTCCAGTTTAAT
241▶ SerPhePhePheLeuSerPheHisIleSerAsnLeuGlnPheAsn
766  TCCTCTCTGGAAGATCCCAGCACCGACTACTACCAAGAGCTGCAG
256▶ SerSerLeuGluAspProSerThrAspTyrTyrGlnGluLeuGln
811  AGAGACATTTCTGAAATGTTTTTGCAGATTTATAAACAAGGGGGT
271▶ ArgAspIleSerGluMetPheLeuGlnIleTyrLysGlnGlyGly
856  TTTCTGGGCCTCTCCAATATTAAGTTCAGGCCAGGATCTGTGGTG
286▶ PheLeuGlyLeuSerAsnIleLysPheArgProGlySerValVal
```

(Continued)

Figure 5 (continued)

```
 901  GTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCAATGTCCAC
 301▶ ValGlnLeuThrLeuAlaPheArgGluGlyThrIleAsnValHis
 946  GACGTGGAGACACAGTTCAATCAGTATAAAACGGAAGCAGCCTCT
 316▶ AspValGluThrGlnPheAsnGlnTyrLysThrGluAlaAlaSer
 991  CGATATAACCTGACGATCTCAGACGTCAGCGTGAGTGATGTGCCA
 331▶ ArgTyrAsnLeuThrIleSerAspValSerValSerAspValPro
1036  TTTCCTTTCTCTGCCCAGTCTGGGGCTGGGGTGCCAGGCTGGGGC
 346▶ PheProPheSerAlaGlnSerGlyAlaGlyValProGlyTrpGly
1081  ATCGCGCTGCTGGTGCTGGTCTGTGTTCTGGTTGCGCTGGCCATT
 361▶ IleAlaLeuLeuValLeuValCysValLeuValAlaLeuAlaIle
1126  GTCTATCTCATTGCCTTGGCTGTCTGTCAGTGCCGCCGAAAGAAC
 376▶ ValTyrLeuIleAlaLeuAlaValCysGlnCysArgArgLysAsn
1171  TACGGGCAGCTGGACATCTTTCCAGCCCGGGATACCTACCATCCT
 391▶ TyrGlyGlnLeuAspIlePheProAlaArgAspThrTyrHisPro
1216  ATGAGCGAGTACCCCACCTACCACACCCATGGGCGCTATGTGCCC
 406▶ MetSerGluTyrProThrTyrHisThrHisGlyArgTyrValPro
1261  CCTAGCAGTACCGATCGTAGCCCCTATGAGAAGGTTTCTGCAGGT
 421▶ ProSerSerThrAspArgSerProTyrGluLysValSerAlaGly
1306  AATGGTGGCAGCAGCCTCTCTTACACAAACCCAGCAGTGGCAGCC
 436▶ AsnGlyGlySerSerLeuSerTyrThrAsnProAlaValAlaAla
1351  ACTTCTGCCAACTTGTGATAA
 451▶ ThrSerAlaAsnLeu••••••
```

Figure 6

```
  1   ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC
  1▶ MetGl n I l ePheVa l Lys Thr LeuThr Gl yLys Thr I l eThr Leu
 46   GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATC
 16▶ Gl uVa l Gl uProSerAspThr I l eGl uAsnVa l LysA l aLys I l e
 91   CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT
 31▶ Gl nAspLys Gl uGl y I l eProProAspGl nGl nArgLeu I l ePhe
136   GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTTCTGACTACAAC
 46▶ A l aGl yLys Gl nLeuGl uAspGl yA rgThr LeuSerAspTyrAsn
181   ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT
 61▶ I l eGl nLys Gl uSer Thr LeuHi s LeuVa l LeuArgLeuArgGl y
226   GGGAGGCACGGTAGTGGTGCATGGCTGTTGCCCGTCTCGCTGGTG
 76▶ Gl yA rgHi s Gl ySer Gl yA l aT rpLeuLeuProVa l Ser LeuVa l
271   AAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCC
 91▶ LysArgLys Thr Thr LeuA l aProAsnThr Gl nThr A l aSer Pro
316   CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCC
106▶ A rgA l aLeuA l aAspSer LeuMet Gl nLeuA l aArgGl nVa l Ser
361   CGAGGATCC
121▶ A rgGl ySer
```

Figure 7

```
  1  ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC
  1▶ Met Gln I l e Phe Va l Lys Thr Leu Thr Gl y Lys Thr I l e Thr Leu
 46  GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATC
 16▶ Gl u Va l Gl u Pro Ser Asp Thr I l e Gl u Asn Va l Lys A l a Lys I l e
 91  CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT
 31▶ Gl n Asp Lys Gl u Gl y I l e Pro Pro Asp Gl n Gl n Arg Leu I l e Phe
136  GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTTCTGACTACAAC
 46▶ A l a Gl y Lys Gl n Leu Gl u Asp Gl y A rg Thr Leu Ser Asp Tyr Asn
181  ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT
 61▶ I l e Gl n Lys Gl u Ser Thr Leu Hi s Leu Va l Leu Arg Leu Arg Gl y
226  GGGAGGCACGGTAGTGGTGCATGGCTGTTGCCCGTCTCGCTGGTG
 76▶ Gl y A rg Hi s Gl y Ser Gl y A l a T rp Leu Leu Pro Va l Ser Leu Va l
271  AAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCC
 91▶ Lys Arg Lys Thr Thr Leu A l a Pro Asn Thr Gl n Thr A l a Ser Pro
316  CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCC
106▶ A rg A l a Leu A l a Asp Ser Leu Met Gl n Leu A l a Arg Gl n Va l Ser
361  CGAGGATCCACAGGTTCTGGTCATGCAAGCTCTACCCCAGGTGGA
121▶ A rg Gl y Ser Thr Gl y Ser Gl y Hi s A l a Ser Ser Thr Pro Gl y Gl y
406  GAAAAGGAGACTTCGGCTACCCAGAGAAGTTCAGTGCCCAGCTCT
136▶ Gl u Lys Gl u Thr Ser A l a Thr Gl n Arg Ser Ser Va l Pro Ser Ser
451  ACTGAGAAGAATGCTGTGAGTATGACCAGCAGCGTACTCTCCAGC
151▶ Thr Gl u Lys Asn A l a Va l Ser Met Thr Ser Ser Va l Leu Ser Ser
496  CACAGCCCCGGTTCAGGCTCCTCCACCACTCAGGGACAGGATGTC
166▶ Hi s Ser Pro Gl y Ser Gl y Ser Ser Thr Thr Gl n Gl y Gl n Asp Va l
541  ACTCTGGCCCCGGCCACGGAACCAGCTTCAGGTTGATAA
181▶ Thr Leu A l a Pro A l a Thr Gl u Pro A l a Ser Gl y • • • • • •
```

Figure 8

```
  1   ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC
  1▶  MetGlnIlePheValLysThrLeuThrGlyLysThrIleThrLeu
 46   GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATC
 16▶  GluValGluProSerAspThrIleGluAsnValLysAlaLysIle
 91   CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT
 31▶  GlnAspLysGluGlyIleProProAspGlnGlnArgLeuIlePhe
136   GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTTCTGACTACAAC
 46▶  AlaGlyLysGlnLeuGluAspGlyArgThrLeuSerAspTyrAsn
181   ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT
 61▶  IleGlnLysGluSerThrLeuHisLeuValLeuArgLeuArgGly
226   GGGAGGCACGGTAGTGGTGCATGGCTGTTGCCCGTCTCGCTGGTG
 76▶  GlyArgHisGlySerGlyAlaTrpLeuLeuProValSerLeuVal
271   AAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCC
 91▶  LysArgLysThrThrLeuAlaProAsnThrGlnThrAlaSerPro
316   CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCC
106▶  ArgAlaLeuAlaAspSerLeuMetGlnLeuAlaArgGlnValSer
361   CGAGGATCCGTGCCCAGCTCTACTGAGAAGAATGCTGTGAGTATG
121▶  ArgGlySerValProSerSerThrGluLysAsnAlaValSerMet
406   ACCAGCAGCGTACTCTCCAGCCACAGCCCCGGTTCAGGCTCCTCC
136▶  ThrSerSerValLeuSerSerHisSerProGlySerGlySerSer
451   ACCACTCAGGGACAGGATGTCACTCTGGCCCCGGCCACGGAACCA
151▶  ThrThrGlnGlyGlnAspValThrLeuAlaProAlaThrGluPro
496   GCTTCAGGTTCAGCTGCCACCTGGGGACAGGATGTCACCTCGGTC
166▶  AlaSerGlySerAlaAlaThrTrpGlyGlnAspValThrSerVal
541   CCAGTCACCAGGCCAGCCCTGGGCTCCACCACCCCGCCAGCCCAC
181▶  ProValThrArgProAlaLeuGlySerThrThrProProAlaHis
586   GATGTCACCTCAGCCCCGGACAACAAGCCAGCCCCGGGAAGTACT
196▶  AspValThrSerAlaProAspAsnLysProAlaProGlySerThr
631   GCTCCACCAGCACACGGTGTTACCTCGGCTCCGGATACCAGGCCG
211▶  AlaProProAlaHisGlyValThrSerAlaProAspThrArgPro
676   GCCCCAGGTAGTACCGCCCCTCCTGCCCATGGTGTCACATCTGCC
226▶  AlaProGlySerThrAlaProProAlaHisGlyValThrSerAla
721   CCGGACAACAGGCCTGCATTGGGTAGTACAGCACCGCCAGTACAC
241▶  ProAspAsnArgProAlaLeuGlySerThrAlaProProValHis
766   AACGTTACTAGTGCCTCAGGCTCTGCTAGCGGCTCAGCTTCTACT
256▶  AsnValThrSerAlaSerGlySerAlaSerGlySerAlaSerThr
811   CTGGTGCACAACGGCACCTCTGCGCGCGCGACCACAACCCCAGCG
271▶  LeuValHisAsnGlyThrSerAlaArgAlaThrThrThrProAla
856   AGCAAGAGCACTCCATTCTCAATTCCCAGCTGATAA
286▶  SerLysSerThrProPheSerIleProSer••••••
```

Figure 9

```
  1   ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC
  1▶  MetGlnIlePheValLysThrLeuThrGlyLysThrIleThrLeu
 46   GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATC
 16▶  GluValGluProSerAspThrIleGluAsnValLysAlaLysIle
 91   CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT
 31▶  GlnAspLysGluGlyIleProProAspGlnGlnArgLeuIlePhe
136   GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTTCTGACTACAAC
 46▶  AlaGlyLysGlnLeuGluAspGlyArgThrLeuSerAspTyrAsn
181   ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT
 61▶  IleGlnLysGluSerThrLeuHisLeuValLeuArgLeuArgGly
226   GGGAGGCACGGTAGTGGTGCATGGCTGTTGCCCGTCTCGCTGGTG
 76▶  GlyArgHisGlySerGlyAlaTrpLeuLeuProValSerLeuVal
271   AAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCC
 91▶  LysArgLysThrThrLeuAlaProAsnThrGlnThrAlaSerPro
316   CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCC
106▶  ArgAlaLeuAlaAspSerLeuMetGlnLeuAlaArgGlnValSer
361   CGAGGATCCGGCTCAGCTTCTACTCTGGTGCACAACGGCACCTCT
121▶  ArgGlySerGlySerAlaSerThrLeuValHisAsnGlyThrSer
406   GCCAGGGCTACCACAACCCCAGCCAGCAAGAGCACTCCATTCTCA
136▶  AlaArgAlaThrThrThrProAlaSerLysSerThrProPheSer
451   ATTCCCAGCCACCACTCTGATACTCCTACCACCCTTGCCAGCCAT
151▶  IleProSerHisHisSerAspThrProThrThrLeuAlaSerHis
496   AGCACCAAGACTGATGCCAGTAGCACTCACCATAGCACGGTACCT
166▶  SerThrLysThrAspAlaSerSerThrHisHisSerThrValPro
541   CCTCTCACCTCCTCCAATCACAGCACTTCTCCCCAGTTGTCTACT
181▶  ProLeuThrSerSerAsnHisSerThrSerProGlnLeuSerThr
586   GGGGTCTCTTTCTTTTTCCTGTCTTTTCACATTTCAAACCTCCAG
196▶  GlyValSerPhePhePheLeuSerPheHisIleSerAsnLeuGln
631   TTTAATTCCTCTCTGGAAGATCCCAGCACCGACTACTACCAAGAG
211▶  PheAsnSerSerLeuGluAspProSerThrAspTyrTyrGlnGlu
676   CTGCAGAGAGACATTTCTGAAATGTTTTTGCAGATTTATAAACAA
226▶  LeuGlnArgAspIleSerGluMetPheLeuGlnIleTyrLysGln
721   GGGGGTTTTCTGGGCCTCTCCAATATTAAGTTCAGGCCAGGATCT
241▶  GlyGlyPheLeuGlyLeuSerAsnIleLysPheArgProGlySer
766   GTGGTGGTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCAAT
256▶  ValValValGlnLeuThrLeuAlaPheArgGluGlyThrIleAsn
811   GTCCACGACGTGGAGACACAGTTCAATCAGTATAAAACGGAAGCA
271▶  ValHisAspValGluThrGlnPheAsnGlnTyrLysThrGluAla
856   GCCTCTCGATATAACCTGACGATCTCAGACGTCAGCGTGAGTGAT
286▶  AlaSerArgTyrAsnLeuThrIleSerAspValSerValSerAsp
901   GTGCCATTTCCTTTCTCTGCCCAGTCTGGGGCTGGGGTGCCAGGC
301▶  ValProPheProPheSerAlaGlnSerGlyAlaGlyValProGly
946   TGGGGCATCGCGCTGCTGGTGCTGGTCTGTGTTCTGGTTGCGCTG
316▶  TrpGlyIleAlaLeuLeuValLeuValCysValLeuValAlaLeu
991   GCCATTGTCTATCTCATTGCCTTGTGATAA
331▶  AlaIleValTyrLeuIleAlaLeu•••••
```

Figure 10

```
  1  ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC
  1▶ Met Gln I l ePhe Va l LysThr LeuThr Gl yLysThr I l eThr Leu
 46  GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATC
 16▶ Gl uVa l Gl uProSer AspThr I l eGl uAsnVa l LysAl aLys I l e
 91  CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT
 31▶ Gl nAspLysGl uGl y I l eProP roAspGl nGl nArgLeu I l ePhe
136  GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTTCTGACTACAAC
 46▶ Al aGl yLysGl nLeuGl uAspGl yA rgThr LeuSer AspTyrAsn
181  ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT
 61▶ I l eGl nLysGl uSer Thr LeuHi s LeuVa l LeuArgLeuArgGl y
226  GGGAGGCACGGTAGTGGTGCATGGCTGTTGCCCGTCTCGCTGGTG
 76▶ Gl yA rgHi s Gl ySer Gl yAl aT rpLeuLeuProVa l Ser LeuVa l
271  AAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCC
 91▶ LysArgLysThr Thr LeuAl aProAsnThr Gl nThr Al aSer P ro
316  CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCC
106▶ A rgAl aLeuAl aAspSer LeuMe t Gl nLeuAl aArgGl nVa l Ser
361  CGAGGATCCCTGGTGCTGGTCTGTGTTCTGGTTGCGCTGGCCATT
121▶ A rgGl ySer LeuVa l LeuVa l CysVa l LeuVa l Al aLeuAl a I l e
406  GTCTATCTCATTGCCTTGGCTGTCTGTCAGTGCCGCCGAAAGAAC
136▶ Va l T yrLeu I l eAl aLeuAl aVa l CysGl nCysArgArgLysAsn
451  TACGGGCAGCTGGACATCTTTCCAGCCCGGGATACCTACCATCCT
151▶ T yrGl yGl nLeuAsp I l ePheP roAl aArgAspThr T yrHi s P ro
496  ATGAGCGAGTACCCCACCTACCACACCCATGGGCGCTATGTGCCC
166▶ Me t Ser Gl uTyrP roThr T yrHi s Thr Hi s Gl yA rgTyrVa l P ro
541  CCTAGCAGTACCGATCGTAGCCCCTATGAGAAGGTTTCTGCAGGT
181▶ ProSer Ser ThrAspArgSer P roT yrGl uLys Va l Ser Al aGl y
586  AATGGTGGCAGCAGCCTCTCTTACACAAACCCAGCAGTGGCAGCC
196▶ AsnGl yGl ySer Ser LeuSer T yrThr AsnP roAl aVa l Al aAl a
631  ACTTCTGCCAACTTGTGATAA
211▶ Thr Ser Al aAsnLeu• • • • • •
```

Figure 11

```
  1  ATGCAGATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTC
  1▶ MetGl n I l ePheVa l LysThr LeuThr Gl yLysThr I l eThr Leu
 46  GAAGTGGAGCCGAGTGACACCATTGAGAATGTCAAGGCAAAGATC
 16▶ Gl uVa l Gl uP roSerAspThr I l eGl uAsnVa l LysA l aLys I l e
 91  CAAGACAAGGAAGGCATCCCTCCTGACCAGCAGAGGCTCATCTTT
 31▶ Gl nAspLysGl uGl y I l eP roP roAspGl nGl nArgLeu I l ePhe
136  GCAGGCAAGCAGCTGGAAGATGGCCGCACTCTTTCTGACTACAAC
 46▶ A l aGl yLysGl nLeuGl uAspGl yA rgThr LeuSerAspTyrAsn
181  ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGT
 61▶ I l eGl nLysGl uSer Thr LeuHi sLeuVa l LeuArgLeuArgGl y
226  GGGAGGCACGGTAGTGGTGCATGGCTGTTGCCCGTCTCGCTGGTG
 76▶ Gl yA rgHi sGl ySer Gl yA l aT rpLeuLeuP roVa l Ser LeuVa l
271  AAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCC
 91▶ LysArgLysThr Thr LeuA l aP roAsnThr Gl nThr A l aSer P ro
316  CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCC
106▶ A rgA l aLeuA l aAspSer LeuMe t Gl nLeuA l aArgGl nVa l Ser
361  CGAGGATCCACAGGTTCTGGTCATGCAAGCTCTACCCCAGGTGGA
121▶ A rgGl ySer Thr Gl ySer Gl yHi sA l aSer Ser Thr P roGl yGl y
406  GAAAAGGAGACTTCGGCTACCCAGAGAAGTTCAGTGCCCAGCTCT
136▶ Gl uLysGl uThr Ser A l aThr Gl nArgSer Ser Va l P roSer Ser
451  ACTGAGAAGAATGCTGTGAGTATGACCAGCAGCGTACTCTCCAGC
151▶ Thr Gl uLysAsnA l aVa l Ser Me t Thr Ser Ser Va l LeuSer Ser
496  CACAGCCCCGGTTCAGGCTCCTCCACCACTCAGGGACAGGATGTC
166▶ Hi sSer P roGl ySer Gl ySer Ser Thr Thr Gl nGl yGl nAspVa l
541  ACTCTGGCCCCGGCCACGGAACCAGCTTCAGGTTCAGCTGCCACC
181▶ Thr LeuA l aP roA l aThr Gl uP roA l aSer Gl ySer A l aA l aThr
586  TGGGGACAGGATGTCACCTCGGTCCCAGTCACCAGGCCAGCCCTG
196▶ T rpGl yGl nAspVa l Thr Ser Va l P roVa l Thr A rgP roA l aLeu
631  GGCTCCACCACCCCGCCAGCCCACGATGTCACCTCAGCCCCGGAC
211▶ Gl ySer Thr Thr P roP roA l aHi sAspVa l Thr Ser A l aP roAsp
676  AACAAGCCAGCCCCGGGAAGTACCGCTCCACCAGCACACGGTGTT
226▶ AsnLysP roA l aP roGl ySer Thr A l aP roP roA l aHi sGl yVa l
721  ACCTCGGCTCCGGATACCAGGCCGGCCCCAGGTAGTACCGCCCCT
241▶ Thr Ser A l aP roAspThr A rgP roA l aP roGl ySer Thr A l aP ro
766  CCTGCCCATGGTGTCACATCTGCCCCGGACAACAGGCCTGCATTG
256▶ P roA l aHi sGl yVa l Thr Ser A l aP roAspAsnA rgP roA l aLeu
811  GGTAGTACAGCACCGCCAGTACACAACGTTACTAGTGCCTCAGGC
271▶ Gl ySer Thr A l aP roP roVa l Hi sAsnVa l Thr Ser A l aSer Gl y
856  TCTGCTAGCGGCTCAGCTTCTACTCTGGTGCACAACGGCACCTCT
286▶ Ser A l aSer Gl ySer A l aSer Thr LeuVa l Hi sAsnGl yThr Ser
```

(Continued)

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Figure 11 (continued)

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 901  GCGCGCGCGACCACAACCCCAGCGAGCAAGAGCACTCCATTCTCA
 301▶ AlaArgAlaThr Thr Thr ProAlaSer LysSer Thr ProPheSer
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 316▶ IleProSer HisHis SerAspThr ProThr Thr LeuAlaSer His
 991  AGCACCAAGACTGATGCCAGTAGCACTCACCATAGCACGGTACCT
 331▶ Ser Thr LysThrAspAlaSer Ser Thr HisHis Ser Thr Val Pro
1036  CCTCTCACCTCCTCCAATCACAGCACTTCTCCCCAGTTGTCTACT
 346▶ ProLeuThr Ser SerAsnHis Ser Thr Ser ProGlnLeuSer Thr
1081  GGGGTCTCTTTCTTTTTCCTGTCTTTTCACATTTCAAACCTCCAG
 361▶ GlyVal Ser PhePhePheLeuSer PheHis IleSerAsnLeuGln
1126  TTTAATTCCTCTCTGGAAGATCCCAGCACCGACTACTACCAAGAG
 376▶ PheAsnSer Ser LeuGluAspProSer ThrAspTyrTyrGlnGlu
1171  CTGCAGAGAGACATTTCTGAAATGTTTTTGCAGATTTATAAACAA
 391▶ LeuGlnArgAspIleSer GluMetPheLeuGlnIleTyrLysGln
1216  GGGGGTTTTCTGGGCCTCTCCAATATTAAGTTCAGGCCAGGATCT
 406▶ GlyGlyPheLeuGlyLeuSerAsnIleLysPheArgProGlySer
1261  GTGGTGGTACAATTGACTCTGGCCTTCCGAGAAGGTACCATCAAT
 421▶ Val Val Val GlnLeuThr LeuAlaPheArgGluGlyThr IleAsn
1306  GTCCACGACGTGGAGACACAGTTCAATCAGTATAAAACGGAAGCA
 436▶ Val HisAspVal GluThr GlnPheAsnGlnTyrLysThr GluAla
1351  GCCTCTCGATATAACCTGACGATCTCAGACGTCAGCGTGAGTGAT
 451▶ AlaSer ArgTyrAsnLeuThr IleSerAspVal Ser Val SerAsp
1396  GTGCCATTTCCTTTCTCTGCCCAGTCTGGGGCTGGGGTGCCAGGC
 466▶ Val ProPheProPheSer AlaGlnSer GlyAlaGlyVal ProGly
1441  TGGGGCATCGCGCTGCTGGTGCTGGTCTGTGTTCTGGTTGCGCTG
 481▶ TrpGly IleAlaLeuLeuVal LeuVal CysVal LeuVal AlaLeu
1486  GCCATTGTCTATCTCATTGCCTTGGCTGTCTGTCAGTGCCGCCGA
 496▶ Ala IleVal TyrLeu IleAlaLeuAlaVal CysGlnCysArgArg
1531  AAGAACTACGGGCAGCTGGACATCTTTCCAGCCCGGGATACCTAC
 511▶ LysAsnTyrGlyGlnLeuAspIlePheProAlaArgAspThr Tyr
1576  CATCCTATGAGCGAGTACCCCACCTACCACACCCATGGGCGCTAT
 526▶ His ProMet Ser GluTyrProThr TyrHis Thr His GlyArgTyr
1621  GTGCCCCCTAGCAGTACCGATCGTAGCCCCTATGAGAAGGTTTCT
 541▶ Val ProProSer Ser ThrAspArgSer ProTyrGluLysVal Ser
1666  GCAGGTAATGGTGGCAGCAGCCTCTCTTACACAAACCCAGCAGTG
 556▶ AlaGlyAsnGlyGlySer Ser LeuSer TyrThrAsnProAlaVal
1711  GCAGCCACTTCTGCCAACTTGTGATAA
 571▶ AlaAlaThr Ser AlaAsnLeu• • • • • •
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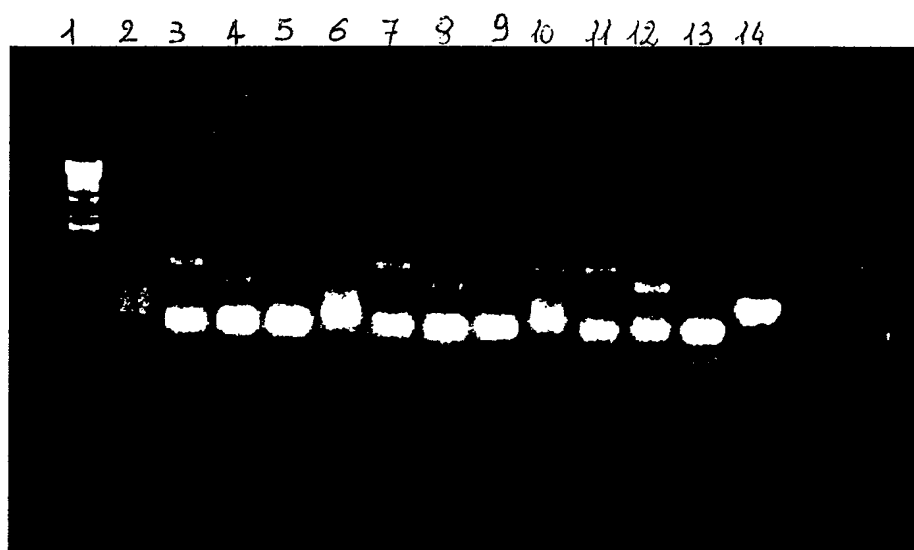
Figure 13

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 101   TTAGGTCACTTAACAAAAAGGAAATTGGGTAGGGGTTTTTCACAGACCGC

 151   TTTCTAAGGGTAATTTTAAAATATCTGGGAAGTCCCTTCCACTGCTGTGT

 201   TCCAGAAGTGTTGGTAAACAGCCCACAAATGTCAACAGCAGAAACATACA

 251   AGCTGTCAGCTTTGCACAAGGGCCCAACACCCTGCTCATCAAGAAGCACT

 301   GTGGTTGCTGTGTTAGTAATGTGCAAAACAGGAGGCACATTTTCCCCACC

 351   TGTGTAGGTTCCAAAATATCTAGTGTTTTCATTTTTACTTGGATCAGGAA

 401   CCCAGCACTCCACTGGATAAGCATTATCCTTATCCAAAACAGCCTTGTGG

 451   TCAGTGTTCATCTGCTGACTGTCAACTGTAGCATTTTTTGGGGTTACAGT

 501   TTGAGCAGGATATTTGGTCCTGTAGTTTGCTAACACACCCTGCAGCTCCA

 551   AAGGTTCCCCACCAACAGCAAAAAAATGAAAATTTGACCCTTGAATGGGT

 601   TTTCCAGCACCATTTTCATGAGTTTTTTGTGTCCCTGAATGCAAGTTTAA

 651   CATAGCAGTTACCCCAATAACCTCAGTTTTAACAGTAACAGCTTCCCACA

 701   TCAAAATATTTCCACAGGTTAAGTCCTCATTTAAATTAGGCAAAGGAATT

 751   CTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTC

 801   ATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGT

 851   GCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATC

 901   CGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGG

 951   AAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGC

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```

Figure 13

2151 TAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGC

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2301 GGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTA

2351 CACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTC

2401 CCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACA

2451 GGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAG

2501 TCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCT

2551 CGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTA

2601 CGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTT

2651 ATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATA

2701 CCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAA

2751 GCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTAT

2801 TTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCAT

2851 AGTTAAGCCAGTATACAATCAATATTGGCCATTAGCCATATTATTCATTG

2901 GTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATACGTTGTAT

2951 CCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCC

3001 ATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGT

3051 CATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTA

3101 AATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAAT

3151 AATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC

3201 AATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTG

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(Continued)

Figure 13 (Continued)

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3451 TCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAAC

3501 GGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGC

3551 GGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAA

3601 CCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAA

3651 GACACCGGGACCGATCCAGCCTCCGCGGCCGGGAACGGTGCATTGGAACG

3701 CGGATTCCCCGTGCCAAGAAAGCTTGTCTAGAACCCGGGAGAGCTCCTGA

3751 GAACTTCAGGGTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTA

3801 TTGTAAAATTCATGTTATATGGAGGGGGCAAAGTTTTCAGGGTGTTGTTT

3851 AGAATGGGAAGATGTCCCTTGTATCACCATGGACCCTCATGATAATTTTG

3901 TTTCTTTCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTTATTTTCT

3951 TTTCATTTTCTGTAACTTTTTCGTTAAACTTTAGCTTGCATTTGTAACGA

4001 ATTTTTAAATTCACTTTTGTTTATTTGTCAGATTGTAAGTACTTTCTCTA

4051 ATCACTTTTTTTTCAAGGCAATCAGGGTATATTATATTGTACTTCAGCAC

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4151 CATATAAATTCTGGCTGGCGTGGAAATATTCTTATTGGTAGAAACAACTA

4201 CATCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACAATGATATACAC

4251 TGTTTGAGATGAGGATAAAATACTCTGAGTCCAAACCGGGCCCCTCTGCT

4301 AACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCT

4351 GGTTGTTGTGCTGTCTCATCATTTTGGCAAAGAATTCACTCCTCAGGTGC

4401 AGGCTGCCTATCAGAAGGTGGTGGCTGGTGTGGCCAATGCCCTGGCTCAC
```

(Continued)

Figure 13 (Continued)

1051 AAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGAT

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1151 AATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTG

1201 TTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAAT

1251 GACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCAT

1301 GACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTG

1351 CGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCT

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1501 CAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACT

1551 CTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGC

1601 AGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATA

1651 AATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGG

1701 CCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCA

1751 GGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCAC

1801 TGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAG

1851 ATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCT

1901 TTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACT

1951 GAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTT

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(Continued)

Figure 13 (Continued)

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4651 GGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGA

4701 GGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAG

4751 AAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTAT

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<211> 579
<212> DNA
<213> human

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<212> DNA
<213> human

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<213> human

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ctctcttaca caaacccagc agtggcagcc acttctgcca acttgtgata a   651

<210> 11
<211> 1737
<212> DNA
<213> human

<400> 11
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<211> 4905
<212> DNA
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<210> 14
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<220>
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<210> 15
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
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<400> 15
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<210> 16
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<220>

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<210> 17
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<210> 18
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<210> 19
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<210> 23
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<210> 25
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<212> DNA
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<220>
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<210> 26
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<220>
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<210> 27
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<210> 28
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<210> 29
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<210> 30
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<212> DNA
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<210> 31
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<213> Artificial Sequence

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<210> 32
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<210> 33
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<210> 35
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